C L A I M S

1. An α-isomaltosylglucosaccharide-forming enzyme which forms a saccharide, having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the linkage at the non-reducing end, by catalyzing the α-glucosyl-transfer from a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end without substantially increasing the reducing power.

2. The α-isomaltosylglucosaccharide-forming enzyme of claim 1, which is substantially incapable of forming dextran and is inhibited by EDTA.

3. The α-isomaltosylglucosaccharide-forming enzyme of claim 1 or 2, wherein said saccharide, having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, is one or more members selected from the group consisting of maltooligosaccharides, maltodextrins, amylodextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

4. An α-isomaltosylglucosaccharide-forming enzyme having the following physicochemical properties:

(1) Action

Forming a saccharide, which has a glucose polymerization degree of at least three and has both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-

1,4 glucosidic linkage other than the linkage at the non-reducing end, by catalyzing the α-glucosyl-transfer from a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end without substantially increasing the reducing power.

(2) Molecular weight

Having a molecular weight of about 74,000 to about 160,000 daltons when determined on SDS-PAGE;

(3) Isoelectric point (pI)

Having an isoelectric point of about 3.8 to about 7.8 when determined on isoelectrophoresis using ampholine;

(4) Optimum temperature

Having an optimum temperature of about 40°C to about 50°C when incubated at a pH of 6.0 for 60 min;

Having an optimum temperature of about 45°C to about 55°C when incubated at a pH of 6.0 for 60 min in the presence of 1 mM Ca^{2+};

Having an optimum temperature of 60°C when incubated at a pH of 8.4 for 60 min; or

Having an optimum temperature of 65°C when incubated at a pH of 8.4 for 60 min in the presence of 1 mM Ca^{2+};

(5) Optimum pH

Having an optimum pH of about 6.0 to about 8.4 when incubated at 35°C for 60 min;

(6) Thermal stability

Having a thermostable region at temperatures of about 45°C or lower when incubated at a pH of 6.0 for 60 min;

Having a thermostable region at temperatures of about 50°C or lower when incubated at a pH of 6.0 for 60 min in the presence of 1 mM Ca^{2+};

Having a thermostable region at temperatures of about 55°C or lower when incubated at a pH of 8.0 for 60 min, and

Having a thermostable region at temperatures of about 60°C or lower when incubated at a pH of 8.0 for 60 min in the presence of 1 mM Ca^{2+}; and

(7) pH Stability

Having a stable pH region at about 4.5 to about 10.0 when incubated at 4°C for 24 hours.

5. The α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 4, which comprises one or more amino acid sequences selected from the group consisting of SEQ ID NO:1, SEQ ID NOs:5 to 7, SEQ ID NOs:11 to 14, and SEQ ID NO:18.

6. The α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 5, which is stabilized and/or activated by Ca^{2+} and Mn^{2+}.

7. The α-isomaltosylglucosaccharide-forming enzyme

of any one of claims 1 to 6, which is a purified or crude enzyme.

8. A process for producing the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 7, which comprises the steps of:

culturing in a nutrient culture medium a microorganism capable of producing the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 6;

and collecting from the resulting culture the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 7.

9. The process of claim 8, wherein said microorganism is of the genus *Bacillus* or *Arthrobacter*.

10. The process of claim 9, wherein said microorganism of the genus *Bacillus* is one selected from the group consisting of *Bacillus globisporus* C9, FERM BP-7143; *Bacillus globisporus* C11, FERM BP-7144; *Bacillus globisporus* N75, FERM BP-7591; and mutants thereof.

11. The process of claim 9, wherein said microorganism of the genus *Arthrobacter* is one selected from the group consisting of *Arthrobacter globiformis* A19, FERM BP-7590; and mutants thereof.

12. A method of α-glucosyl-transferring reaction, which comprises a step of contacting the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1 to 7 with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-

1,4 glucosidic linkage as a linkage at the non-reducing end.

13. The method of claim 12, wherein a saccharide-transferred product is formed by the α-glucosyl-transferring reaction in the presence of one or more acceptors selected from the group consisting of D-glucose, D-xylose, L-xylose, D-galactose, D-fructose, D-mannose, D-arabinose, D-fucose, D-psicose, L-sorbose, methyl-α-glucopyranoside, methyl-β-glucopyranoside, N-acetylglucosamine, trehalose, isomaltose, isomaltotriose, cellobiose, gentibiose, glycerol, maltitol, lactose, sucrose, and L-ascorbic acid.

14. A method for forming α-isomaltosyl-glucosaccharide, which comprises a step of contacting the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1 to 7 with a solution, comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, to effect α-glucosyl-transferring reaction.

15. The method of claim 14, wherein said saccharide is one selected from the group consisting of maltooligosaccharides, maltodextrins, amylodextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

16. A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by contacting both the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1 to 7 and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide

having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor.

17. The cyclotetrasaccharide or the saccharide composition of claim 16, wherein said saccharide having a glucose polymerization degree of at least two is one selected from the group consisting of maltooligosaccharides, maltodextrins, amylodextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

18. A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by:

contacting both the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1 to 7 and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end to obtain a solution comprising said cyclotetrasaccharide along with other saccharide(s); and

subjecting said solution to column chromatography using a strong-acid cation exchange resin,

wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the

resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor.

19. The cyclotetrasaccharide or the saccharide composition of any one of claims 16 to 18, which contains at least 30% (w/w), on a dry solid basis, of the cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}.

20. The cyclotetrasaccharide or the saccharide composition of any one of claims 16 to 19, which is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystalline powder, or crystalline solid.

21. The cyclotetrasaccharide or the saccharide composition of claim 20, wherein said crystal is one or more members selected from the group consisting of a crystalline cyclotetrasaccharide, penta- or hexa-hydrate; a crystalline cyclotetrasaccharide, monohydrate; and an anhydrous crystalline cyclotetrasaccharide, which all have the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}.

22. The cyclotetrasaccharide or the saccharide composition of claim 20 or 21, wherein said crystal is prepared by crystallizing in an aqueous solution without using any organic solvent.

23. The cyclotetrasaccharide or the saccharide composition of any one of claims 16 to 22, wherein said saccharide comprising cyclotetrasaccharide is one which

comprises cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} and one or more saccharides selected from the group consisting of glucose, maltose, and a saccharide having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the non-reducing end.

24. A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by culturing a microorganism in a nutrient culture medium comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, said microorganism being capable of producing both the α-isomaltosylglucosaccharide-forming enzyme as claimed in any one of claims 1 to 7 and an α-isomaltosyl-transferring enzyme which specifically hydrolyzes the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety and which transfers the released α-isomaltosyl moiety to an acceptor.

25. The cyclotetrasaccharide or the saccharide composition, wherein said saccharide having a glucose polymerization degree of at least two is one selected from the group consisting of maltooligosaccharides, maltodextrins, amylodextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

26. The cyclotetrasaccharide or the saccharide composition as claimed in claim 24 or 25, wherein said

microorganism is of the genus *Bacillus* or *Arthrobacter*.

27. The cyclotetrasaccharide or the saccharide composition as claimed in claim 26, wherein said microorganism of the genus *Bacillus* is one selected from the group consisting of *Bacillus globisporus* C9, FERM BP-7143; *Bacillus globisporus* C11, FERM BP-7144; *Bacillus globisporus* N75, FERM BP-7591; and mutants thereof.

28. The cyclotetrasaccharide or the saccharide composition as claimed in claim 26, wherein said microorganism of the genus *Arthrobacter* is one selected from the group consisting of *Arthrobacter globiformis* A19, FERM BP-7590; and mutants thereof.

29. The cyclotetrasaccharide or the saccharide composition as claimed in any one of claims 24 to 28, which is obtainable by:

contacting the resulting culture or the saccharide obtainable from the culture with one or more enzymes selected from the group consisting of α-amylase, β-amylase, glucoamylase, and α-glucosidase; and

treating the resultant mixture with one or more purification methods selected from the group consisting of decoloration, desalting, fractionation by column chromatography, separation with a membrane, fermentation with a microorganism, and alkaline treatment.

30. The cyclotetrasaccharide or the saccharide composition of claim 16, 18 or 24, wherein said α-isomaltosyl-transferring enzyme has the following physicochemical properties:

(1) Action

Forming a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} from a saccharide having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the above linkage;

(2) Molecular weight

Having a molecular weight of about 82,000 to about 136,000 daltons when determined on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE);

(3) Isoelectric point (pI)

Having a pI of about 3.7 to about 8.3 when determined on isoelectrophoresis using ampholine;

(4) Optimum temperature

Having an optimum temperature of about 45°C to about 50°C when incubated at a pH of 6.0 for 30 min;

(5) Optimum pH

Having an optimum pH of about 5.5 to about 6.5 when incubated at 35°C for 30 min;

(6) Thermal stability

Having a thermostable range at temperatures

of about 45°C or lower when incubated at a pH of 6.0 for 60 min;

(7) pH Stability

Having a stable pH range at about 3.6 to about 10.0 when incubated at 4°C for 24 hours.

31. A process for producing a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which comprises a step of contacting with a solution of gelatinized and/or liquefied starch both the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1 to 7 and an α-isomaltosyl-transferring enzyme, which specifically hydrolyzes the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme and which transfers the released α-isomaltosyl moiety to an acceptor.

32. The process of claim 31, wherein said solution of gelatinized and/or liquefied starch has a DE (dextrose equivalent) of not higher than 20.

33. The process of claim 31 or 32, wherein said solution of gelatinized and/or liquefied starch is contacted with the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 7, α-isomaltosyl-transferring enzyme, and cyclomaltodextrin glucanotransferase, and optionally one or more enzymes selected from the group consisting of α-amylase, β-amylase, glucoamylase, and α-glucosidase.

34. The process of claim 31, 32 or 33, which further contains one or more purification methods selected from the group consisting of decoloration, desalting, fractionation by column chromatography, separation with a membrane, fermentation treatment using microorganisms, and decomposition by an alkaline treatment.

35. The process of any one of claims 31 to 34, which contains at least 30% (w/w), on a dry solid basis, of a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}.

36. The process of any one of claims 31 to 35, wherein said cyclotetrasaccharide or said saccharide composition comprising the same is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystalline powder, or crystalline solid.

37. The process of claim 36, wherein said crystal is prepared by crystallizing in an aqueous solution without using any organic solvent.

38. The process of claim 31 or 33, wherein said α-isomaltosyl-transferring enzyme has the following physicochemical properties:

(1) Action

Forming a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} from a saccharide having a glucose polymerization degree of at least three and

having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the above linkage;

(2) Molecular weight

Having a molecular weight of about 82,000 to about 136,000 daltons when determined on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE);

(3) Isoelectric point (pI)

Having a pI of about 3.7 to about 8.3 when determined on isoelectrophoresis using ampholine;

(4) Optimum temperature

Having an optimum temperature of about 45°C to about 50°C when incubated at a pH of 6.0 for 30 min;

(5) Optimum pH

Having an optimum pH of about 5.5 to about 6.5 when incubated at 35°C for 30 min;

(6) Thermal stability

Having a thermostable range at temperatures of about 45°C or lower when incubated at a pH of 6.0 for 60 min;

(7) pH Stability

Having a stable pH range at about 3.6 to about 10.0 when incubated at 4°C for 24 hours.

39. A composition comprising the cyclotetrasaccharide

of any one of claims 16 to 30, having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}, or a saccharide composition comprising the same.

40. The composition of claim 39, wherein said cyclotetrasaccharide or said saccharide composition comprising the same is used as a saccharide having one or more properties of sweetness, substantial non-fermentability, low cariogenicity, low calorific value, osmosis-pressure controllability, filler-imparting ability, gloss-imparting ability, moisture-retaining ability, viscosity-imparting ability, syneresis-preventing ability, solidification-preventing ability, inclusion ability, flavor-retaining ability, stability, ability to prevent crystallization of other saccharides, retrogradation-preventing ability, protein-denaturation-preventing ability, lipid-deterioration-preventing ability, acid tolerance, heat resistance, and ability of hardly causing the amino carbonyl reaction; or used directed to one or more uses as a sweetener, low cariogenic food material, low caloric food material, taste-imparting agent, flavor-imparting agent, quality-imparting agent, syneresis-preventing agent, flavor-imparting agent, stabilizer, discoloration-preventing agent, filler-imparting agent, inclusion agent, and material for pulverization.

41. The composition of claim 39 or 40, which is in the form of a food product, cosmetic, or pharmaceutical.

42. The composition of claim 39 or 40, which comprises at least 0.1% (w/w), on a dry solid basis, of the cyclotetrasaccharide.